

# **Chapter 6**

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## **CONDUCTING AN EPIDEMIOLOGIC INVESTIGATION**

- 1) What is Epidemiology?**
- 2) Conducting an Epidemiologic Investigation**
- 3) Steps in an Epidemiologic Investigation**
- 4) Submission of Clinical Specimens to the  
State Laboratory Institute**

# CONDUCTING AN EPIDEMIOLOGIC INVESTIGATION

### Introduction

Epidemiologic investigation is an important part of the complete foodborne illness investigation that also includes environmental (see Chapter 7) and laboratory investigations (see Section 4 of this chapter and Appendix B). Each part of the investigation compliments the other and team work and open communication is of utmost importance.

The purpose of the epidemiologic investigation is to identify a problem, collect data, formulate and test hypotheses. It involves the collection and analysis of more facts or data to determine the cause of illness and to implement control measures to prevent additional illness.

This chapter addresses epidemiology, the steps involved in an epidemiologic investigation, and laboratory submission of clinical specimens.

## 1) What is Epidemiology?

A text book definition of epidemiology is the study of the **distribution** and **determinants** of disease **frequency** in human populations. It is the collection and analysis of data to determine whether an association may exist between one or more exposures and the occurrence of disease. In practice, epidemiologists often employ statistics and probability to look at who gets sick or injured and why. In a sense, epidemiology is as old as medicine itself. Hippocrates suggested, in the fifth century B.C., that the development of human disease might be related to the external as well as the personal environment of an individual.

John Snow, a British physician is frequently considered the “father” of epidemiology. His investigations of cholera in London in the 1840’s-1850’s drew together all three components of the definition of epidemiology (frequency, distribution and determinants of disease). When a cholera outbreak occurred in London, Snow determined that cases occurred most frequently in specific neighborhoods of the city that used water supplied by one company. Snow canvassed the involved neighborhood to determine the source of water for each household that had a case of cholera. Snow charted the frequency and

distribution of cases and was able to discover possible causes and determinants of infections. At one point cases were mapped to the supply of one particular water pump, Snow had the handle of the implicated water pump removed. The approach used by Snow is still used today and is outlined in Section 3, “Steps In An Epidemiologic Investigation” below.

## 2) Conducting an Epidemiologic Investigation

Epidemiologic investigations are usually conducted in outbreak situations. The main reasons for conducting an epidemiologic investigation are:

- to determine the cause of an outbreak, and
- to implement control measures to prevent additional illness.

A questionnaire is often solicited to assist the investigator in developing better hypotheses about the etiologic agent’s identity, source and transmission. The investigators interview ill and well persons, and calculate and compare rates of illness in both groups. They make time, place, and person associations and calculate the probability that a food was the responsible vehicle.

The investigator incorporates results from epidemiological associations and the environmental and laboratory investigations, and uses these data in forming and testing hypotheses. Careful development of epidemiologic inferences coupled with persuasive clinical and laboratory evidence will almost always provide convincing evidence of the source and mode of spread of a disease. In situations where food and stool testing are negative, the cause of an outbreak is often implicated by epidemiological association.

In addition to the above, epidemiologic investigations also serve as a teaching tool. By carrying out the following steps you will gain an understanding of the systematic, logical approach an epidemiologist or “disease detective” follows in an investigation.

It is often unclear when to conduct a full epidemiologic investigation. There is usually no question when you are notified about a large number of people getting ill at approximately the same time after eating at the same establishment or attending the same event. However, uncertainty arises when sporadic complaints are reported. You will need to consider whether the reports indicate that the affected cases are all suffering from the same illness and whether there is any evidence of an association between them. This underscores the need to follow-up (i.e., determine the validity of and initiate further action if necessary) on every complaint you receive. You may find that single complaints are actually related to an outbreak.

- Refer to Chapter 4, Sections 3 and 4 for more information on what information to collect and how to collect it.

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- Refer to Chapter 5, Sections 2 and 3 for more information on handling single complaints.

When you are notified of an incident in which illness has resolved and no new cases have been identified, your decision to conduct an epidemiologic investigation should be based on an assessment of what you will gain from it. As stated above, an investigation always serves as a learning tool. But, if you do not have the resources (time, personnel, etc.), it may not be warranted to conduct a full investigation. Rather, you should ensure that appropriate control measures have been implemented to prevent future outbreaks.

This is especially true of home-based foodborne outbreaks. In many instances, the illness is confined to a finite number of people in a discrete time period. In addition, you are notified after the fact when there is little material left for testing and people have recovered. You should review food preparation techniques with the responsible parties and use the opportunity to educate them on proper food handling and preparation methods.

**NOTE:** Investigation of an outbreak of foodborne illness is a team effort where each member has an essential role to perform. In some instances the team may include a number of individuals at the local level (public health nurse, sanitarian, laboratory, health agent) as well as the Working Group on Foodborne Illness Control (WGFIC) at the state level. At times, there may be only one person involved at the local level. **Whatever your circumstances, it is important to remember that the WGFIC is available for guidance and assistance throughout each step of your investigation.** Phone numbers for the Working Group are listed below.

### MDPH Working Group on Foodborne Illness Control

Division of Food and Drugs (617) 983-6712	For policy and technical assistance with the environmental investigation such as conducting a HACCP risk assessment, initiating enforcement actions and collecting food samples. On-site investigation assistance is often available for larger outbreaks.
Division of Epidemiology and Immunization (617) 983-6800	For technical assistance with the epidemiologic investigation such as obtaining medical histories, coordinating stool specimen submissions and developing questionnaires. On-site investigation assistance is often available for larger outbreaks.
Division of Diagnostic Laboratories (617) 983-6616	For technical assistance with the collection protocol for food and clinical specimens.

### 3) Steps in an Epidemiologic Investigation

The following steps need to be taken in all epidemiologic investigations.

1. Confirm the existence of an epidemic or an outbreak.
2. Confirm the diagnosis.
3. Determine the number of cases.
4. Orient the data in terms of time, person and place.
5. Develop a hypothesis.
6. Compare the hypothesis with the established facts.
7. Execute control and preventive measures.
8. Write a written report.

**NOTE:** It is important to note that while the above list of steps is in a particular order, they do not necessarily have to be carried out in that order. In fact, several steps may be put into action simultaneously. However, confirming the diagnosis and the establishment of the existence of an outbreak always deserve early attention.

**NOTE:** Depending on staffing, resources and time, you may not be able to cover all the steps or cover them thoroughly. As stated previously, the WGFIC is available for guidance and assistance. (Telephone numbers for the WGFIC are listed on the previous page.)

**Step 1. Confirm the existence of an epidemic or an outbreak.** What is an epidemic or an outbreak? In Chapter 5, Section 5, an outbreak of foodborne illness is defined as two or more persons experiencing a similar illness after ingestion of a common food OR different food in a common place. An outbreak may also be defined as a situation when the observed number of cases unaccountably exceeds the expected number. However, with certain foodborne illnesses such as botulism or chemical poisoning, a single case would elicit an in-depth epidemiological and environmental investigation.

To determine if there is an outbreak, you can compare the current number of cases (incidence) with past levels of the same disease over a similar time period. If the number is unusually large or unexpected for the given place and time, you may have an outbreak. For example, in June of 1996, there was an outbreak of salmonellosis in a town west of Boston. When five cases of gastrointestinal illness were identified among patrons of a fast food restaurant, the local board of health (LBOH) immediately notified the Division of Food and Drugs. The LBOH clearly identified this as an unusual occurrence that led to the initiation of the investigation.

An outbreak may not always manifest itself in an obvious manner as above. Outbreaks dispersed over a broad geographic area, with few cases in any one jurisdiction, are much more difficult to detect locally. This underscores the importance of establishing and

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maintaining a surveillance system discussed in Chapter 4. By maintaining a surveillance system and reporting to the MDPH in a timely manner, an outbreak dispersed over a broad geographic area may then be recognized at the state level.

When trying to confirm an outbreak, it is important to rule out other causes for increases in numbers of cases. For example, you might notice that several cases of *E. coli* O157:H7 have been reported to you over the past month. When you compare the numbers with cases recorded for the same month last year, you notice an increase. Then you remember that *E. coli* O157:H7 was recently made a reportable disease and this could be “surveillance artifact.” That would be an artificial increase, and not necessarily a cause for alarm. Media attention to other outbreaks of the same disease tend to heighten public awareness and can lead to an increased number of cases being reported.

**Step 2. Confirm the diagnosis.** This is done by obtaining appropriate specimens for laboratory study and obtaining clinical histories.

Laboratory study is done by standard methods, (e.g., blood tests, stool culture). Be wary of verbal reports of cases of hepatitis A. Insist on obtaining laboratory evidence of positive IgM anti-HAV (IgM hepatitis A antibody). Other evidence to support the diagnosis, (e.g., a lab-confirmed case in a contact) can sometimes be used in lieu of laboratory results. (Information on submitting clinical specimens is discussed in Section 4 of this chapter.) In some instances, there will be outbreaks of unknown etiology, and there will be no laboratory results to confirm the diagnosis. Cases or outbreaks of diseases of unknown etiology are just as valid as those with known etiologies.

**NOTE:** Laboratory identification of a pathogen can validate the hypothesis and perhaps allow easier implementation of control and preventive measures. **Therefore, time is of the essence when requesting and collecting clinical and food specimens.**

- Refer to Section 4 of this chapter for information on submission of clinical specimens.
- Refer to Chapter 7, Section 1 and Appendix B for more information on submission of food specimens.

Whether the etiology is known or not, the investigator must still characterize the illness by interviewing ill persons, family members or physicians. This can be done through phone calls, informal interviews, or a more formal survey that will be discussed further in Step 3 - “Determine the number of cases.” **Remember, this information is confidential and should be shared with only those individuals involved in the investigation.** (See Chapter 4, Section 5 for more information on confidentiality.)

To initially assist in the organization of data, a good starting point can be the creation of a “line listing” table. Case names and numbers are listed down the left hand column, and the

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heading row at the top of the table should contain pertinent information such as the case's age, sex, onset time, and symptoms. This type of organization permits a simple means for comparison of many characteristics, at one time, for possible patterns, similarities, or associations. Further on in the investigation you may want to conduct a survey or questionnaire (discussed in Step 3 below).

### Example of a Line Listing Table

#	Name	Age	Sex	Onset Date	Onset Time	Symptoms
1	Mary	32	F	5/4/97	1:00 PM	Diarrhea, abd. cramps
2	Bob	25	M	5/4/97	1:30 PM	Diarrhea
3	Carol	26	F	5/4/97	10:15 AM	Diarrhea, nausea
4	Mark	18	M	5/3/97	11:30 PM	Diarrhea, abd. cramps

**Step 3. Determine the number of cases (ill people).** This helps to get an idea of the magnitude of the problem. Determination of case numbers is based on creating a **case definition**. A case definition is a set of criteria for deciding whether an individual should be classified as a case. The case definition places boundaries on who is considered a case, so the investigation does not include those with illnesses unrelated to the outbreak.

The common elements of a case definition include information on symptoms, laboratory results, time, place and person.

**a) Symptoms:** People with the same illness do not always have the same symptoms, but they will experience similar ones. It is important to remember that the symptoms of some foodborne illnesses can mimic other foodborne diseases. The following list of symptoms can be used as a “general rule of thumb” for determining the incubation period and possible etiologic agent:

- chemical poisoning symptoms, (e.g., vomiting) usually start within 1 hour of ingestion;
- nausea and vomiting usually start within 6 hours of ingestion;
- cramps and diarrhea usually start between 6-20 hours after ingestion;
- and diarrhea, chills, fever usually start between 12-72 hours after ingestion.

An example of a case definition that is commonly used for foodborne illness outbreaks without a known cause is: an individual who attended a specific event and then experienced diarrhea or a combination of two to three other gastrointestinal symptoms within a specified time after the event.

**b) Laboratory results:** If you are fortunate enough to have a laboratory confirmed diagnosis, this will make the task of defining a case much easier. You may want to consider notifying the laboratories in your jurisdiction that an outbreak exists and ask them to notify you of additional cases of the illness under investigation. **Note: during**

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**an outbreak of foodborne illness, efforts should be made to send all specimens and/or isolates to the State Laboratory Institute (SLI) for further identification, confirmation and to assure coordination of the investigation.** (See Sections 4-B and 4-C of this chapter for more information on what testing is done at the SLI.)

**c) Time:** If there appears to be a common meal involved, then the time between consumption of that meal and the onset of symptoms provides an indication of the incubation period. The incubation period and symptoms are helpful in determining which illnesses should be considered as possible causes of the outbreak and thus may facilitate decision-making regarding what types of laboratory tests should be run. As with symptoms, incubation periods can vary among individuals; therefore, be sure to offer a range of time when considering an incubation period. For example, if you are investigating a salmonella outbreak, you may want to include, as cases, those persons who experienced symptoms consistent with the case definition anywhere from 6 - 72 hours after the meal in question.

**d) Place:** When there is a common meal involved, you already know the place. But sometimes the only information available may be that cases are occurring in several different locations over the same time period. It is only after more information becomes available that the case definition will become more specific as to the location of the outbreak.

**e) Person:** The outbreak may or may not take place within a particular group of people. Therefore, characteristics such as age, sex, occupation, ethnic group, social affiliations or function attendance greatly assist in qualifying the case definition.

Your initial case definition should be general so that potential cases are not left out. Once you have more information about the outbreak, you can refine the case definition to “weed out” extraneous cases. Once you have the case definition in place, decide how to find additional cases, (i.e., routine methods versus more intensive methods). Do you feel comfortable relying on telephone reporting from physicians? Or do you feel the need to actively search for cases from area physicians or area laboratories, use local media or enlist the help of the local hospital?

### **The Questionnaire/Survey**

A common method of finding cases, organizing and analyzing data is to conduct a questionnaire or survey among the population you believe to be at risk, (e.g., attendees of a wedding). A questionnaire that targets specific questions about foods eaten and symptoms experienced is a valuable epidemiologic tool. A questionnaire is solicited to those ill and well, associated with the incident, and assists in developing better hypotheses about the etiologic agent’s identity, source, including the means and time of transmission.

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Key questions to consider when developing a questionnaire

- What are the demographic characteristics of the individual? (name, age, sex, occupation, home and work addresses, phone numbers)
- Was the individual exposed to the suspected source and when?
- What are the symptoms, date of onset, their order of occurrence and duration?
- What medical treatment has been sought and received?
- Is there a diagnosis or laboratory results?
- Who else has been exposed to a case during his or her infectious period? (secondary contacts)
- What foods were consumed in the last 72 hours or other appropriate time frame, before the time of onset. It is also important to interview and obtain food histories from those who ate the same suspect food and did not get sick.

These questions are intended as a guide. They will require modification to fit the particular circumstances surrounding the investigation. Questionnaires can be designed for personal or telephone interviews by the investigator (nurse, sanitarian, health agent, etc.). A self-administered form can also be conducted through the mail, but the response rate can be lower, and responses can take a long time.

<p><b>NOTE:</b> An example of a foodborne illness questionnaire/survey can be found at the end of this chapter (Attachment 6.5).</p>
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For those who have computer capabilities, there is a computer software program called EPI INFO which can be used to develop questionnaires and analyze data. (The software is free. You can obtain a copy by contacting the Division of Epidemiology and Immunization at (617) 983-6800 or via the Internet at: [www.cdc.gov](http://www.cdc.gov)). For more information about when you should send out a questionnaire or about EPI INFO, contact the Division of Epidemiology and Immunization at (617) 983-6800.

Another useful tool for collecting this information or to initially spot an outbreak is the *Bacterial/Parasitic Gastroenteritis Case Report Form*. (Further information on *case report forms* can be found in Chapter 4, Section 4-B.) As discussed in Chapter 4, timeliness of reporting is important to ensure that control measures to prevent additional cases are implemented as soon as possible.

**Step 4. Orient the data in terms of TIME, PLACE, and PERSON.** The purpose of data orientation or epidemiological characterizations is to arrange all incoming data so it means something. The investigator is searching for common associations based on TIME, PLACE, and PERSON to strengthen or amend current hypotheses. A common method of data orientation is plotting, on a graph, the cases by time of symptom onset to get an **epidemic curve**.

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**NOTE:** An **epidemic curve** is a graph that depicts the association of the time of illness onset of all cases that are associated with the outbreak. It helps to determine whether the outbreak originated from a common source or person to person. Time is plotted on the horizontal axis and the number of cases plotted on the vertical axis.

From the line listing and/or survey described above (Steps 2 and 3), you will have collected information on the characteristics of the ill persons (age, sex, occupation, exposures to specific foods or other items). Very often, simply by knowing these descriptive aspects, the diagnosis and then plotting an **epidemic curve**, the source, mode of transmission and who is at risk can be determined. Once the population at risk has been determined, appropriate control measures can be targeted.

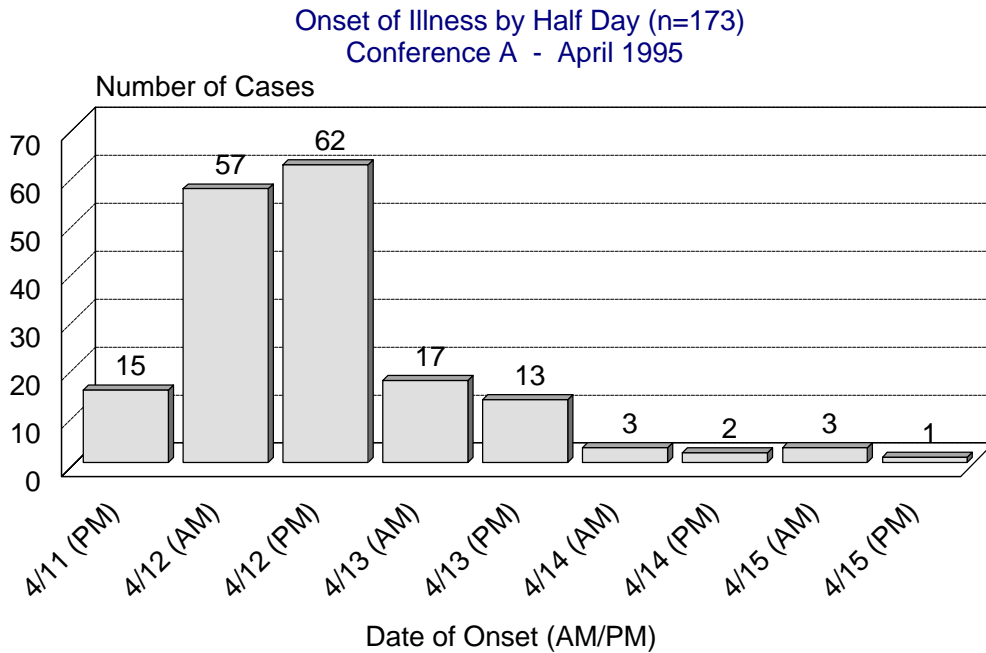
The shape of the epidemic curve may suggest what kind of outbreak is occurring. A *common-source* or *point-source outbreak* looks different than a *propagated-source* or *person-to-person outbreak* and a *continual source outbreak*. Definitions of these kinds of outbreaks, and an example of each epidemic curve are found below. Epidemic curves are also useful when communicating to lay persons (consumers, restaurant operators, etc.) the nature and magnitude of the outbreak spread.

**NOTE:** The following pages contain definitions and examples of the different kinds of outbreaks:

- Common-Source or Point-Source Outbreak
- Propagated-Source Outbreak or Person-to-Person Outbreak
- Continual-Source Outbreak

**Common-Source or Point-Source Outbreak.** An outbreak of disease or illness in which susceptible individuals are exposed simultaneously to one source of infection. For example: guests at a wedding reception. The epidemic curve for this type of outbreak is characterized by a sharp rise to a peak followed by a decline usually less abrupt than the rise. See Example 6.1 below.

### Example 6.1 Common-Source Outbreak Epidemic Curve



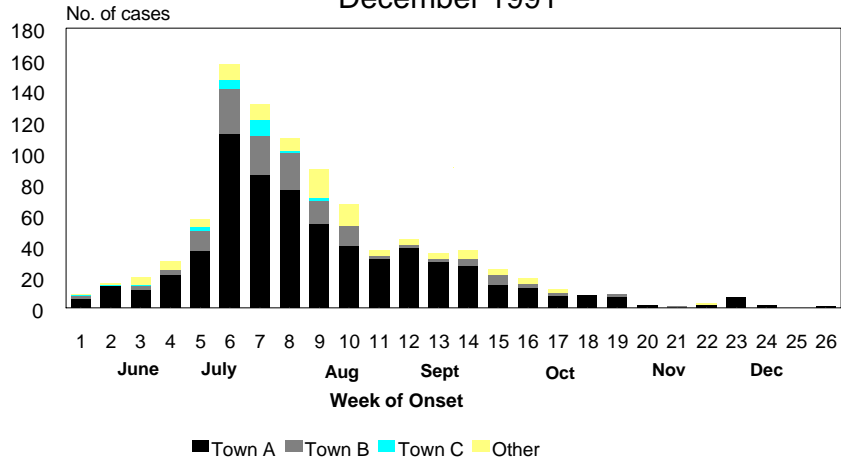
Source: MDPH, Working Group on Foodborne Illness Control, 1995

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**Propagated-Source Outbreak or Person-to-Person Outbreak.** An outbreak of disease or illness that is spread from one person to another rather than from a single source. For example: a community-wide outbreak of shigellosis. The epidemic curve for this type of outbreak is characterized by a relatively slow, progressive rise. The curve will continue for the duration of several incubation periods of the disease. For example: a shigellosis outbreak in western MA lasted about six months. See Example 6.2 below.

### Example 6.2 Propagated-Source Outbreak Epidemic Curve

#### SHIGELLOSIS Hampden County Area, June - December 1991

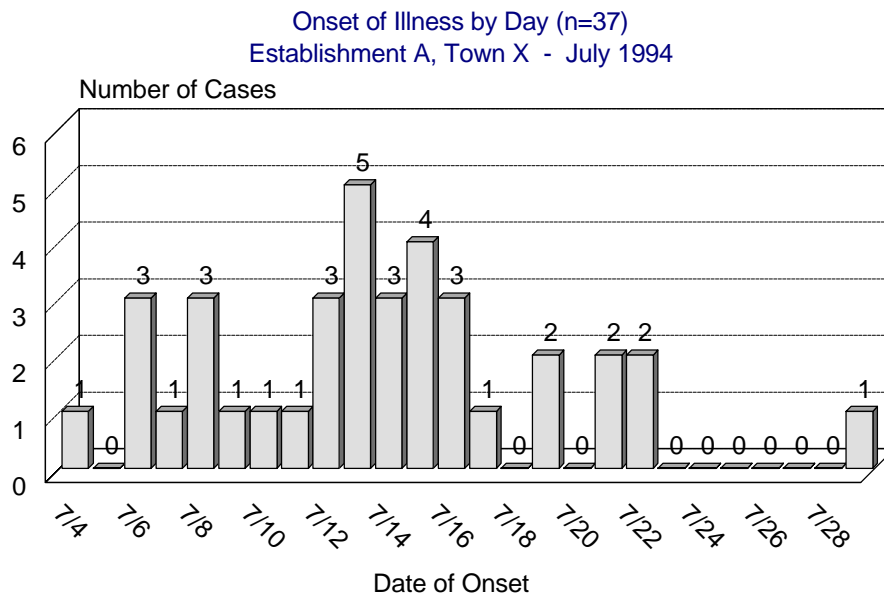


N = 927 with known date of onset  
(Cases reported as of 12/27/91)

Source:  
MDPH, Working Group on Foodborne Illness Control, 1991

**Continual-Source Outbreak.** An extended outbreak of disease or illness caused by a source that continues to be contaminated. For example: an outbreak where food is continuously contaminated by an infected food handler. The epidemic curve for this type of outbreak is characterized by continual peaks over time (e.g., weeks, months). The peaks may not be as dramatic as a common-source epidemic curve, and the outbreak may not be as obvious. See Example 6.3 below.

**Example 6.3**  
**Continual-Source Epidemic Curve**



Source: MDPH, Working Group on Foodborne Illness Control, 1994

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**NOTE:** Remember, if at any time throughout the entire investigation, an ongoing, potentially hazardous source of illness is discovered, recommendations should be decided and acted upon. Regulatory actions may also need to be taken.

**Step 5. Develop a hypothesis that explains the specific exposure(s) that may have caused the disease (and test this by appropriate statistical methods).** Using the information gathered from the previous steps, consider the possible source(s) from which the disease may have been contracted. One example of a simple hypothesis is: the cases became ill after sharing a common meal.

As stated in Step 4, very often, simply by knowing the descriptive aspects, the diagnosis and then plotting an **epidemic curve**, the source, mode of transmission and who is at risk can be determined. Once the population at risk has been determined, appropriate control measures can be targeted. This descriptive aspect of the epidemiological investigation is what is most often carried out at the local level.

To test or prove your hypothesis, you would want to apply more analytical techniques, such as statistical testing. This is often carried out by an epidemiologist at the state level (or in collaboration with the state), and for the purposes of this manual, will be referred to below and not discussed in detail.

Often in a foodborne illness outbreak, food-specific attack rates (AR) are calculated. Attack rates are used to determine if one or more food items were responsible for causing the illness. The food that caused the problem shows a higher attack rate in persons who ate the food than in those who did not. The AR is usually expressed in percent. It represents the proportion of ill persons observed due to a specific exposure or event.

You may have heard of other terms: “odds ratio, relative risk, and p-value.” These are some of the statistical tests that can be used to test a hypothesis. Many of these tests are automatically calculated by computer programs like EPI INFO, although they can be done by hand.

**NOTE:** Refer to Example 8.3 - Outbreak Report in Chapter 8 for an example of an investigation where more advanced analytical techniques were employed. (Note the various tables and graphs at the end of this report.)

**NOTE:** If you have computer capabilities and/or are interested in learning more about analytical epidemiology and statistical testing, contact the Division of Epidemiology and Immunization. Additionally, please refer to the references at the end of this chapter.

**Step 6. Compare the hypothesis with the established facts and draw conclusions.** For example, based on evidence gathered, you have a hypothesis that the salad was the vehicle of transmission in a salmonella outbreak. You then need to ask yourself how the salad became contaminated with salmonella and could this be verified with the results of the environmental investigation. In other words, are your epidemiologic results plausible and consistent with other investigational findings? For instance, salad is not usually a food that harbors salmonella. However, it can become contaminated when ill or infected food handlers prepare the salad without adequate handwashing or use of gloves. Compare your hypothesis to the results of the environmental investigation. Did the inspector note how the salad was made and served? Was it possible for this scenario to have happened? Some of the questions that need to be addressed to make sure that your hypothesis is not only statistically sound, but makes sense in the real world are:

- Could your hypothesized events actually have happened?
- Is your hypothesis consistent with the environmental aspects of the investigation? (See Chapter 7 for more information on environmental investigations.)
- Is it likely the vehicle of transmission identified became contaminated with the organism that has been isolated?

**NOTE:** Not all outbreaks have a resolution. In fact, it is rare when everything comes together and a cause can be definitively determined. Do not be discouraged. Careful development of epidemiologic inferences coupled with persuasive clinical and environmental evidence will almost always provide convincing evidence of the source and mode of spread of a disease. In most cases, there will be enough evidence to present a plausible hypothesis.

**Step 7. Execute control and preventive measures.** Before initiating any control measures, think about the effectiveness, timeliness, costs, available resources, personnel requirements and possible ramifications of proposed actions. Are the recommendations realistic for the establishment involved? For example, will they be able to install the new dishwasher or the 3-bay sink that was recommended? If not, what are the alternatives?

**NOTE: Be advised that some control measures should be implemented very early in an outbreak investigation.** For example, removal of ill food handlers or the embargo, recall or destruction of contaminated food items should be implemented immediately, if necessary.

In addition, all corrective actions must be verified by the LBOH to ensure that steps to reduce or eliminate the hazards have actually occurred. See Chapter 7, Section 3-Steps 4 and 5 for additional information on control and preventive measures.

**Step 8. Write a report.** After analysis of epidemiologic and environmental data, conclusions should be summarized in a report. This is one of the most important steps in

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the outbreak investigation. Not only does the report detail your agency's efforts, but identifies a potential source(s) of the outbreak and suggests control measures to prevent future illness.

- See Chapter 8 for detailed information on writing a report. Sample reports are also included in Chapter 8.

### 4) Submission of Clinical Specimens to the State Laboratory Institute

Clinical specimens (blood, feces, etc.) were mentioned in the previous section under Step 2, "Confirming The Diagnosis." Laboratory identification of a pathogen can validate the hypothesis and perhaps allow easier implementation of control and preventive measures. Increased certainty results if statistical association is combined with isolation of a pathogen from the ill person and the implicated food. This evidence is almost certain to be irrefutable. **Therefore, time is of the essence when requesting and collecting clinical and food specimens.**

- Refer to Appendix B for more information on submitting food specimens.

#### A. Role of the State Laboratory

The Division of Diagnostic Laboratories (DDL) is part of the State Laboratory Institute (SLI) of the MDPH located at 305 South Street in Jamaica Plain, Massachusetts. The DDL is the state reference laboratory, where hospitals and other laboratories send specimens or isolates for confirmation and serotyping. In addition to reference laboratory activities, the DDL examines implicated food and clinical specimens (in outbreak and non-outbreak situations) to identify the organism or extraneous materials responsible for human illness. Specimens are submitted by local boards of health, other public agencies and health care providers.

The two units within the DDL that conduct foodborne illness-related testing are:

- the Enteric Lab (stool testing) and
- the Food Microbiology Lab (often referred to as the Food Lab).

The Environmental Chemistry Lab may be involved in situations that involve suspected chemical poisonings or for the testing of naturally occurring toxins. In outbreak situations, LBOHs can coordinate food and clinical specimen submissions with the DDL to ensure that all specimens (e.g., food handlers, patrons, implicated foods) are handled in a coordinated fashion.

### B. What is accepted for testing?

#### Feces and Food

The two specimens considered most appropriate for foodborne illness-related testing are **feces** and **food**. Food specimen submission is addressed in Appendix B.

#### Other Specimens

**Urine** is not a usual specimen for culture although the Enteric Lab does receive isolates (usually from hospital labs) from urine specimens of *Salmonella*, *Shigella* and *E. coli* O157:H7 for identification or serotyping. If the board of health should receive notification from the Enteric Lab of a positive pathogen from a urine specimen, follow-up should include a stool specimen. If the case is a food handler, the employee still must submit at least one negative stool specimen for clearance to return to work (with the exception of *S. typhi* which is three negative stool specimens). See *105 CMR 300: Reportable Diseases and Isolation and Quarantine Requirements*.

**Blood** is an acceptable specimen when typhoid or botulism is suspected (see Section 4-F on more information on botulism testing), or the clinician requests blood testing for another reason. Blood tests for hepatitis A are usually performed through the individual's private medical provider, and are not performed at the State Laboratory Institute.

### C. What tests are performed on fecal specimens?

Routine cultures:

- *Campylobacter* species
- *Salmonella* species
- *Shigella* species
- *Vibrio* species
- *Yersinia* species
- *E. coli* O157:H7, also known as Enterohemorrhagic colitis or EHEC

Other cultures and tests performed upon special request (if symptoms of illness are consistent) include those for:

- *Clostridium perfringens*
- *Bacillus cereus*
- *Staphylococcus aureus*
- *Clostridium botulinum* (see Section 4-F)

<p><b>NOTE:</b> The DDL does not examine specimens for ova and parasites (e.g., <i>Giardia lamblia</i>, <i>Cryptosporidium parvum</i>, <i>Cyclospora cayetanensis</i>). If there is a need for ova and parasite testing (e.g., food handlers, individuals without health insurance), arrangements can be made through the SLI for specimens to be sent to a contract laboratory.</p>
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The DDL does not perform viral isolation on stool specimens. In special circumstances, the Centers for Disease Control and Prevention (CDC) in Atlanta may be able to offer laboratory assistance and conduct viral testing on fresh stool specimens. **If there is a situation that warrants this service, contact the Division of Epidemiology and Immunization (617-983-6800) or the DDL (617-983-6600) for assistance.**

### D. Turnaround Times on Specimens (specimens submitted directly to SLI)

The following table details the minimum time to complete enteric testing from receipt of sample to test result. (This does not include weekend days.)

**Table 6.4 - Stool Testing Turnaround Times**

<b>Species</b>	<b>Positive (minimum hrs)</b>	<b>Negative (minimum hrs)</b>
Campylobacter	48	72
Salmonella	48	72
Yersinia	96	48
Shigella	48	24
Vibrio	96	48
<i>E. coli</i> O157:H7	48	24
<i>C. perfringens</i>	72	48
<i>Bacillus cereus</i>	72	24
<i>S. aureus</i>	72	48

### E. Procedure for Stool Sample Collection and Submission

- 1) The Enteric Laboratory currently provides the following fecal specimen collection kits:
  - Transport for *Salmonella sp.*, *Shigella sp.*, *Yersinia sp.* The kit contains a clear plastic bottle with a *white* label and a green top.
  - Transport for *Campylobacter sp.* only. The kit contains a clear plastic bottle with an *orange* label and a green top.
  - The combined outfit which contains both types of transport bottles.

**NOTE:** 1) The preferred method for submitting specimens for *E. coli* O157:H7 is a fresh stool in a sterile container placed on wet ice submitted to the laboratory as quickly as possible. If this is not feasible, contact the Enteric Laboratory at (617) 983-6609 for further instruction.

2) Instructions for the submission of specimens suspected of containing *Vibrio species*. can be obtained by calling the Enteric Laboratory at (617) 983-6609.

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**NOTE:** All enteric culture kits now provided by the SLI have clearly labeled “expiration dates” on them. Older kits had a date stamped on the outer container. This date is not an expiration date but indicates the date the kits were made. Kits generally last for about 10 years. It is not necessary to discard older kits. The kit can be used as long as the transport medium (liquid solution inside) has not turned yellow. If the solution has turned yellow, **DO NOT USE**. Return the unused kit and obtain a new one.

### 2) When to collect clinical specimens

- Diagnosis of most foodborne diseases can be made more easily when etiologic agents are isolated from clinical specimens of ill persons. **Encourage ill persons to submit stool specimens while they are still experiencing symptoms or as soon as is practical thereafter. Pathogens or toxins may remain in the intestinal tract for only a short time after illness onset.**
- Collect stool specimens prior to antibiotic treatment. **NOTE: A repeat sample may need to be submitted if the patient was on antibiotics when the initial culture was taken. This often happens if the patient is a food handler and needs clearance to return to work.**

### 3) How much to collect

- A sample, the size of a dime, should be placed on the paddle in the transport medium. The medium stabilizes the specimens and prevents overgrowth of normal flora. If the stool is liquid, transfer no more than 4 ml of specimen to the container. **DO NOT OVERFILL.**
- Take care not to contaminate feces with urine.
- A container such as a bed pan or plastic wrap can be placed over the toilet for easier fecal collection.

### 4) Rectal swabs

Swabs are not usually recommended for testing because the sample size is too small (exception, see Section 4-F, “Botulism Testing”). If a rectal swab is the only available sample, care should be taken to insert the swab past the anal sphincter muscle to obtain a representative fecal specimen. Transfer the swab to the appropriate transport container, rotate the swab in the medium, press the swab vigorously against the side of the container, break or cut off the handle and include swab with container.

### 5) Label each specimen bottle with:

- patient name
- physician name and address (or local board of health name and address)
- date of specimen collection

### 6) Complete the *Enteric Culture Requisition* form that is found inside the container. It must include the following:

- patient name, address, date of birth, sex

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- provider information (board of health, private physician, etc.) Name and address are needed to report results.
- occupation, (e.g., food handler, daycare provider, etc.)
- history of recent travel outside the U.S.
- history of shellfish consumption
- whether the patient is a “contact” to a known case or whether this is a “release” or clearance specimen
- note whether a particular food establishment is involved or if part of a known outbreak

### 7) Packaging

- **To prevent leakage, tighten cover of transport bottle completely.**
- Place transport bottle into inner metal container.
- Place *Enteric Culture Requisition* form between the inner metal and outer cardboard mailing container.

### 8) Delivery

- Mail the specimen using first class postage or
- Hand deliver to:

DDL

Enteric Laboratory - Fourth Floor

State Laboratory Institute

305 South Street

Jamaica Plain, MA 02130

### NOTE:

- If there are any questions, contact the Enteric Laboratory at (617) 983-6609.
- To obtain stool kits, contact the DDL secretary (617) 983-6603.
- Appendix E includes a form entitled *Instructions For Submitting Enteric Specimens*. This two-page form contains abbreviated instructions on the information in this section.

## F. Botulism Testing

Request for testing for possible botulism cases should come directly to the Division of Epidemiology and Immunization. Since botulism testing is very involved, i.e., not a simple blood test (see below), physicians need to contact the Division of Epidemiology and Immunization directly to discuss the situation. The epidemiologist collects a clinical history and a food history and discusses with the physician the patient’s symptoms and other test results. After the information is collected, the epidemiologist and the physician determine whether or not botulism testing is appropriate. If a decision to test is made, the other members of the WGFIC (DDL and DFD) and the LBOH will be contacted to coordinate specimen collection and laboratory preparation.

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If the case is highly suspect, antitoxin arrangements must be made. Stool samples need to be collected **before** antitoxin is administered. Approval for release of antitoxin must be obtained from the CDC. Antitoxin is only administered to adult patients. This antitoxin, of equine origin, may cause serum sickness in approximately 20% of treated persons. Antitoxin is not used for infants because of the lack of evidence of its benefit.

### **Specimen Collection**

The primary and best specimen for foodborne botulism testing is stool. A quantity of at least 25 grams (1 oz.) can be collected into any sterile container (there is no special kit). With infants less than one year of age, it is acceptable to submit rectal swabs. Multiple swabs are usually collected to obtain as much stool as possible.

The stool must be kept fresh (no preservatives) on wet ice and sent to the SLI as quickly as possible. If the sample cannot be delivered on the same day as collection, it can be kept refrigerated. Ideally, the lab should receive the specimen early in the day.

The secondary specimen is serum (not whole blood). A minimum of 10 cc (5 cc for infants less than one year of age) needs to be collected and delivered to the SLI on wet ice. Again, it can be refrigerated if it cannot be delivered on the same day as collected. **NOTE:** The likelihood of demonstrating toxin in serum is much less than in stool.

### **Stool testing**

The stool is tested by two methods. The first is a mouse bioassay or the mouse neutralization test. Extracts from the stool specimen that may contain botulinum toxin are injected into live mice. The mice are paired, with one being the control and the other receiving the extract with antitoxin. The mice are then observed for neurologic symptoms and death. Testing may take a few days or several weeks for a definitive answer. A second method is by culture to isolate the organism, *Clostridium botulinum*. This process can take from 4-6 days. Culture of toxin-producing clostridium is confirmed by the mouse neutralization test.

### **Serum testing**

Serum is tested by the mouse neutralization method (described above).

### **Food testing**

Coordination for pickup and testing of food samples is arranged between the Division of Epidemiology and Immunization, DFD, LBOH, the family or the restaurant involved. Food is tested by the methods described above.

## **G. Reporting Results**

Written reports on all positive results are sent to:

- the CDC in Atlanta,
- the board of health where the patient lives (always),

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- other submitters as noted on the *Enteric Culture Requisition* form (e.g., the patient's physician or a hospital lab),
- the board of health handling an outbreak,
- the Bureau of Communicable Disease Control, and
- the DDL files.

Written reports on all negative results are sent to:

- the board of health where patient lives (if the patient is a release or contact, if BOH is listed as submitter on *Enteric Culture Requisition* form or if BOH requests result),
- other submitters as noted on the *Enteric Culture Requisition* form (e.g., the patient's physician or a hospital lab),
- the board of health handling an outbreak, and
- the DDL files.

## References

Beaglehole, R., Bonita, R., Kjellstrom, T. *Basic Epidemiology*. Geneva: WHO, 1993.

Committee on Communicable Diseases Affecting Man, Food Subcommittee. *Procedures to Investigate Foodborne Illness*. Fourth Edition, Des Moines, Iowa: International Association of Milk, Food and Environmental Sanitarians, Inc., 1988.

FDA, Division of Human Resource Development, State Training Branch. *Principles and Concepts For Investigating Foodborne Illness*. U.S. Government Printing Office, 1994.

Gregg, M. B. *Oxford Textbook of Public Health*. Holland: Oxford University Press, 1985.

Hennekens, C. and Buring, J. E. *Epidemiology in Medicine*. Toronto: Little, Brown and Company, 1987.

Mausner, J. and Kramer, S. *Epidemiology An Introductory Text*. Philadelphia: W. B. Saunders Company, 1985.

## ATTACHMENT 6.5 Sample Questionnaire/Survey

Board of Health Letterhead here.

July 25, 1996

The Massachusetts Department of Public Health in conjunction with the XXXXX Board of Health is investigating an outbreak of gastrointestinal illness which occurred among the attendees of a business conference held at Establishment A on July 19, 1996. Please complete these questions **ONLY IF YOU ATTENDED** the meeting. Your completion of the following questions, **EVEN IF YOU DID NOT HAVE ANY SYMPTOMS**, will greatly assist us in our efforts to identify the source of this illness. **All information which you provide will be kept strictly confidential and used solely for the purposes of this investigation.** Please return the completed questionnaire to the XXXXX Board of Health at the address or fax below. Thank you for your assistance.

**RETURN TO:**

Board of Health  
(123) 456-7890  
Fax: (123) 456-7781

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**PLEASE DO NOT LEAVE ANY QUESTIONS BLANK**

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DATE COMPLETED: \_\_\_\_/\_\_\_\_/\_\_\_\_

1. LAST NAME \_\_\_\_\_ FIRST NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_ TOWN \_\_\_\_\_

STATE \_\_\_\_\_ ZIP CODE \_\_\_\_\_ SEX: ☐M ☐F AGE \_\_\_\_\_

PHONE: (\_\_\_\_) - \_\_\_\_ - \_\_\_\_\_

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2. Please mark any of the following **symptoms** that you have had **SINCE ATTENDING** the meeting on July 19, 1996?

☐ NONE \*(IF NONE, PLEASE MARK NONE AND GO TO QUESTION #10)

<input type="checkbox"/> Chills	<input type="checkbox"/> Nausea	<input type="checkbox"/> Vomiting	<input type="checkbox"/> Abdominal Cramps	
<input type="checkbox"/> Fever	<input type="checkbox"/> Muscle Aches	<input type="checkbox"/> Headaches	<input type="checkbox"/> Loss of Appetite	<input type="checkbox"/>
<input type="checkbox"/> Fatigue	<input type="checkbox"/> Dizziness	Highest Temp: _____		
<input type="checkbox"/> Diarrhea: # Episodes of diarrhea per day: _____				
Type of diarrhea:				
<input type="checkbox"/> Soft stool				
<input type="checkbox"/> Watery Diarrhea				
<input type="checkbox"/> Bloody Diarrhea				
Other Symptoms: _____				

3. **What day** did your symptoms begin? ☐ Friday, 7/19/96  
☐ Saturday, 7/20/96  
☐ Sunday, 7/21/96  
☐ Monday, 7/22/96  
☐ Other \_\_\_\_\_

4. **What time** did your symptoms begin? TIME: \_\_\_\_\_:\_\_\_\_\_ ☐ AM ☐ PM

5. **How long** did your symptoms last? ☐ HOURS: # \_\_\_\_\_ (Choose either hours or days)  
☐ DAYS: # \_\_\_\_\_

6. Did you seek **medical care** for your symptoms? ☐ YES ☐ NO  
If YES, name of doctor? \_\_\_\_\_ Phone # / Town

7. Were you **hospitalized** for your symptoms? ☐ YES ☐ NO  
If YES, name of hospital? \_\_\_\_\_

8. Did you provide a **stool sample** for testing? ☐ YES ☐ NO  
Results of the test? \_\_\_\_\_

9. Did any family/household members **who did not attend the meeting** experience similar symptoms following your illness? ☐ YES ☐ NO

If YES, # ILL \_\_\_\_\_ DATE(S): \_\_\_\_\_

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**10.** Have **you** recently had any diarrhea, vomiting, or other symptoms **before the meeting?**

☐ YES

☐ NO

If YES, DATE(S): \_\_\_\_\_

**11.** Did you eat any food(s) or drink any beverage(s) at the meeting held on July 19, 1996 at Establishment A? ☐ YES ☐ NO

If YES, what time did you eat?

TIME: \_\_\_\_:\_\_\_\_ ☐ AM ☐ PM

**12.** Please mark YES OR NO to indicate whether you consumed the following items:

Turkey ☐ YES ☐ NO

Ham ☐ YES ☐ NO

Salami ☐ YES ☐ NO

Cheese ☐ YES ☐ NO

Bread Rolls ☐ YES ☐ NO

Sandwich Condiments:

Lettuce ☐ YES ☐ NO

Onion ☐ YES ☐ NO

Tomato ☐ YES ☐ NO

Other \_\_\_\_\_

Pickle ☐ YES ☐ NO

Mayonnaise ☐ YES ☐ NO

Mustard ☐ YES ☐ NO

Potato Salad ☐ YES ☐ NO

Tuna Salad ☐ YES ☐ NO

Coleslaw ☐ YES ☐ NO

Tossed Green Salad ☐ YES ☐ NO

Type of Dressing: ☐ French ☐ Italian ☐ No Dressing

Broccoli soup ☐ YES ☐ NO

Chocolate Cake ☐ YES ☐ NO

Carrot Cake ☐ YES ☐ NO

Danish ☐ YES ☐ NO

Sliced Fruit ☐ YES ☐ NO

Type of fruit: ☐ Pineapple ☐ Canteloupe ☐ Honeydew Melon

Water ☐ YES ☐ NO

Ice ☐ YES ☐ NO

Coffee ☐ YES ☐ NO

Tea ☐ YES ☐ NO

Cream ☐ YES ☐ NO

Milk ☐ YES ☐ NO

Soda ☐ YES ☐ NO

Juice ☐ YES ☐ NO

Other Beverages or Food: \_\_\_\_\_

THANK YOU FOR RESPONDING TO THESE QUESTIONS